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Evolutionary consequences of multidriver environmental change in an aquatic primary producer

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Climate change is altering aquatic environments in a complex way, and simultaneous shifts in many properties will drive evolutionary responses in primary producers at the base of both freshwater and marine ecosystems. So far, evolutionary studies have shown how changes in environmental drivers, either alone or in pairs, affect the evolution of growth and other traits in primary producers. Here, we evolve a primary producer in ninety-six unique environments with different combinations of between one and eight environmental drivers to understand how evolutionary responses to environmental change depend on the identity and number of drivers. Even in multidriver environments, only a few dominant drivers explain most of the evolutionary changes in population growth rates. Most populations converge on the same growth rate by the end of the evolution experiment. However, populations adapt more when these dominant drivers occur in the presence of other drivers. This is due to an increase in the intensity of selection in environments with more drivers, which are more likely to include dominant drivers. Concurrently, many of the trait changes that occur during the initial short-term response to both single and multidriver environmental change revert after about 450 generations of evolution. In future aquatic environments, populations will encounter differing combinations of drivers and intensities of selection, which will alter the adaptive potential of primary producers. Accurately gauging the intensity of selection on key primary producers will help in predicting population size and trait evolution at the base of aquatic food webs.

multiple environmental drivers | adaptation | microbial evolution | *Chlamydomonas*

Introduction

A growing body of evidence from experiments shows that functional traits in aquatic primary producers can be altered by evolution in the face of global change, which has to date been explored in terms of drivers such as temperature (1, 2) or CO₂ levels (3). These studies investigate the responses of primary producers to single aspects of global change (4), and the results are often used to understand how changes to the biological component of nutrient cycling, including air-water carbon exchange, will be impacted (5, 6). Recent short-term studies show that in multidriver environments, the majority of the organismal response is often explained by one or two drivers (7, 8). We call these dominant drivers (7). This study investigates how the evolutionary responses to dominant environmental drivers depends on the other drivers present. This helps link patterns of complex (multidriver) environmental change to the evolutionary potential of primary producers, and informs the design of future experiments. We do this using an evolution experiment that disentangles the effects of driver number and identity on trait evolution in multidriver environments in a single celled alga.

A small number of experiments have investigated the plastic and evolutionary responses to multiple drivers thus far (7, 9–12). These studies consistently show that both plastic and evolutionary responses to pairs of drivers differ from responses to either single driver. In the short-term, the effect of multidriver environments on plastic responses (changes in phenotype in response to an environmental cue that does not require change in the genetic

composition of the population) can be understood through the mode of interaction of the drivers (7, 13). However, we previously showed that when more than three drivers co-occur, average plastic responses in growth are explained by dominant drivers (7), because of either small interactions between non-dominant, or zero-sum interactions between drivers.

Aquatic primary producers will evolve under global change, due to their rapid cell division rates, and high standing genetic variation and ability to generate genetic variation (4, 14, 15). We do not currently have an empirically-supported, general understanding of how natural selection differs between single- and multidriver environments. However, complex environmental change will be common in aquatic environments, with combinations and intensities of drivers having significant regional variation (8), so it is vital that we understand the joint contributions of the identity, number, and intensity of drivers to trait evolution in primary producers. Two non-exclusive mechanisms could cause natural selection to act differently in cases of complex (multidriver) versus simple (single driver) environmental change. First, if the number of independent traits under selection increases as the number of drivers in the environment increases, pleiotropic interactions could limit adaptation in complex environments more than in simple ones (16). Second, if selection intensity increases as the number of drivers increases, then the probability of population extinction increases with the number of drivers, but surviving populations will adapt more and more rapidly in environments with more drivers. These two mechanisms have different ecological consequences. If differences in evolutionary responses are mainly due to differences in pleiotropy under multidriver change,

Significance

Our understanding of how primary producers at the base of aquatic ecosystems respond to complex environmental change currently depends on studies using few environmental drivers, or scenarios where drivers covary. However, we lack a general understanding of evolution in multidriver environments. We evolve a microbial primary producer in ninety-six different multidriver environments and find that evolutionary responses in growth are largely driven by a few drivers, but that the intensity of selection is, on average, higher in multidriver environments. Functional traits (cell size, chlorophyll content) often revert to ancestral values during adaptation in multidriver environments. This expands the framework for understanding how microbial primary producers evolve under global change, and the potential ramifications for their function in aquatic ecosystems.

Reserved for Publication Footnotes

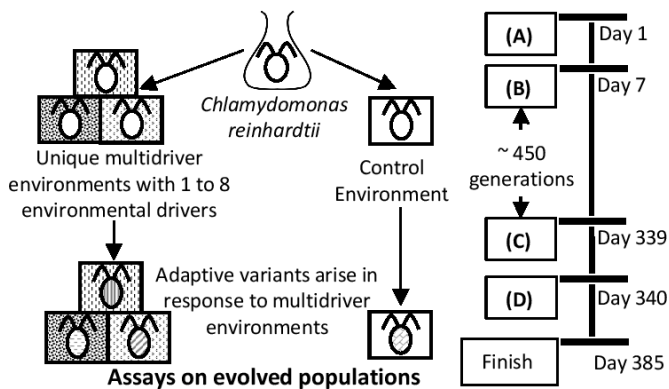


Fig. 1. Schematic diagram illustrates the experimental design of the study. (A) The founding population was established from one colony of *C. reinhardtii*, grown from a single cell. (B) The founding population was grown for one week under control conditions, then split into ninety-six different regimes (square boxes) with one to eight environmental drivers (regimes are shown as different pattern backgrounds), and a control environment (white background). (C) Populations evolved in each regime for ninety-five transfers. This provides enough time for adaptive variants to arise and increase in frequency. (D) After ninety-five transfers, the multidriver-evolved populations were assayed in their regime and the control environment. The control populations were assayed in all test regimes.

we expect that shifts in communities result mainly from changes in interactions between groups, with less trait evolution within groups than predicted based on single-driver experiments. In contrast, if the intensity of selection increases with the number of drivers, we expect that in addition to shifts in the taxonomic composition of communities, there be changes in functional trait values within groups (17). Trait evolution in primary producers can in turn affect how food webs and aquatic nutrient cycles are impacted (18). Finally, if there are increased pleiotropic limitations as well as stronger selection in multidriver environments, then we expect more local extinctions and less trait evolution in surviving populations in multidriver relative to single driver environments.

We use experimental evolution to measure how the number and identity of environmental drivers affect evolution in an initially isogenic population. Using many driver combinations allows us to disentangle the effects of driver number and identity on trait evolution, though some driver combinations are unrealistic (19, 20). The strength of this approach is that it builds a general understanding of how natural selection acts in multidriver environments. However, the model system and environments suitable for this experimental design mean that our findings cannot be applied directly to the immediate debate on how marine life will respond to global change; the fundamental insights gained here must be translated to the appropriate systems and environments, which can be done in smaller targeted studies. Replicate clonal populations of *Chlamydomonas reinhardtii* were grown in ninety-six unique environments (each unique environment is referred to as a regime) with up to eight simultaneous drivers including elevated temperature, elevated CO₂, periodic UVB exposure, reduced light intensity, reduced phosphate, acidification, reduced nutrients, and the addition of herbicide, for ~450 asexual generations. See Fig. 1 for a schematic, and Table S1 for drivers in each regime. See SI Appendix for a discussion of drivers and driver intensities. Discussions of plastic responses to single drivers was previously published in (7). Because the populations were initially isogenic, genetic variation in the evolved populations is from novel mutations or other heritable changes (epigenetic mutations or transgenerational plasticity). Since the role of primary producers in aquatic systems is determined not only by their population growth rates, but also by their trait values (21, 22),

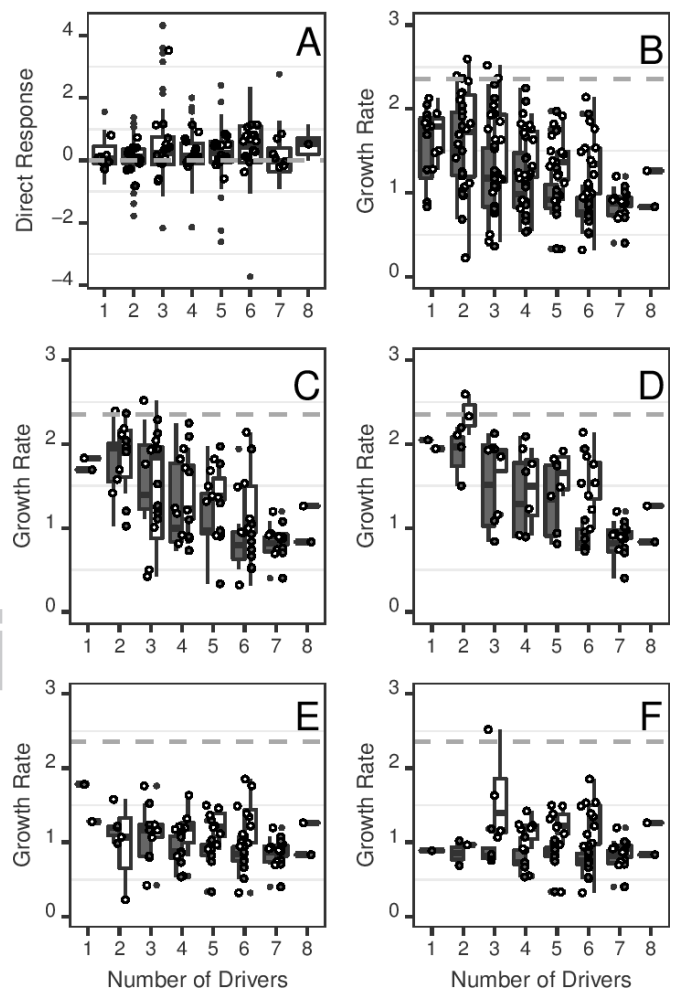


Fig. 2. The response of evolved populations under increasing numbers of drivers. Boxes show the (A) direct response to selection measured as the average number of cell divisions (d^{-1}) relative to control populations assayed in the same regime. Open circles show the average of evolved populations within in each regime. The dashed line indicates that there is no difference between the growth rate of the evolved control and the multidriver-evolved populations, in the same selection regime. Average cell divisions (d^{-1}) of evolved populations assayed in (B) all regimes (C) regimes with elevated CO₂ (D) regimes with elevated temperature, (E) regimes with reduced phosphate and (F) regimes with herbicide. White symbols show multidriver populations assayed in their selection regimes and grey symbols show control populations assayed in the same regimes. The dashed line (B-F) shows the average growth rate of control populations in the control environment.

we measured evolutionary change in cell size, and a commonly-used proxy for primary production (chlorophyll) (23). Cell size is a “master trait” that constrains several organismal characteristics and biotic interactions for single-celled organisms, such as growth and metabolic rates (17), nutrient affinity (17), light absorption affinity (24), and predation (25).

Results

Following ~450 generations of evolution in 96 regimes, we compared the endpoints of evolution by measuring the growth rates of the evolved populations. Populations evolved in multidriver environments all reached similar growth rates by the end of the experiment. However, the small amount of variation in evolved growth rate was explained by a few individual drivers, regardless of which other drivers were present. These dominant drivers were elevated CO₂ (Fig. 2C; $F_{1,77} = 5.454$, $P = 0.022$), elevated temperature (Fig. 2D; $F_{1,78} = 10.042$, $P = 0.002$), reduced phosphate

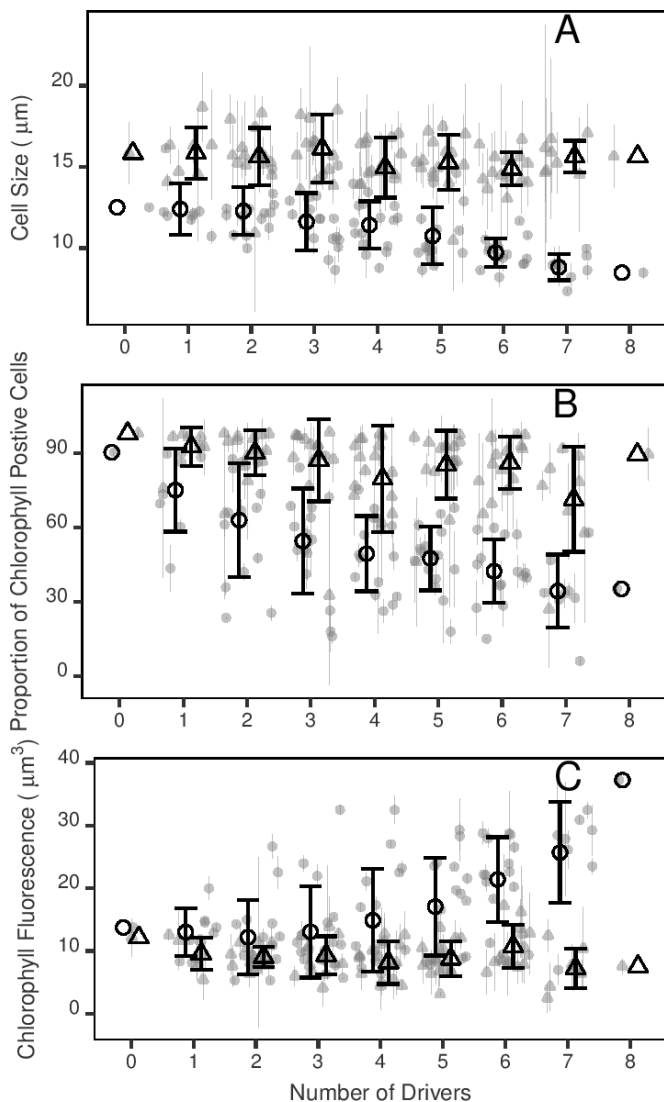


Fig. 3. Trait values of *C. reinhardtii* before and after evolution in multidriver environments. Changes in (A) cell size, (B) proportion of chlorophyll positive cells and (C) chlorophyll autofluorescence per cell volume ($1/\mu\text{m}^3$) in populations of *C. reinhardtii*. In all panels, black symbols show the response (\pm SD) for a given number of drivers and grey symbols show the average growth rate (\pm SD) for each regime. Circles represent the plastic response and triangles represent the evolved response

(Fig. 2E; $F_{1,77} = 20.686$, $P < 0.0001$), and herbicide (Fig. 2F; $F_{1,77} = 22.036$, $P < 0.0001$). Here, CO_2 increased growth rates, while the other dominant drivers decreased growth (Fig. S4). The selection regimes themselves explained only 5% of the variation in growth rates of populations in their selection regimes. Thus, the dominant drivers drive growth rate evolution. The overall effect of the number of environmental drivers in a selection regime on evolved population growth rate is not significant (Fig. 2B, white boxplots; $F_{1,73} = 0.043$, $P = 0.837$) and the small amount of present was explained by variation among evolved replicate populations within regimes (43%). Thus, increasing the number of drivers in multidriver environments does not constrain the endpoint of evolution on average, at least in terms of population growth rates.

Evolved populations have undergone ~450 generations of evolution in their selection regimes (see Fig. 1, time C). The direct response to selection compares the population growth rate of a population evolved in a given regime with the plastic response of a

control population to that same regime. This measures the difference in plastic and evolutionary responses to an environment, and estimates net adaptive change over the experiment. A positive response to selection indicates that evolution increases growth rates beyond the plastic response, and a negative direct response to selection indicates that evolution slows growth relative to the plastic response.

While populations converge on similar growth rates, the direct response to selection is larger when there are more drivers in selection regimes, so that populations in environments with more drivers evolve more to arrive at the same endpoint. This is because in environments with more drivers, populations tend to have lower initial growth rates (Fig. 2B-F), which indicates stronger selection. This is consistent with extinctions occurring in seven and eight driver environments (Fig. S1, Fig. 2B). Across all regimes, variation in the initial drop in growth rate explains variation in the direct response to selection, regardless of the number of drivers (Fig. 2A; *effect of plastic response on direct response*, $F_{1,341} = 69.356$, $P < 0.0001$). The larger direct response to selection in regimes with more drivers is thus attributable to stronger selection in these regimes. Intermediate timepoints were not characterized, so we cannot draw conclusions about the timing of adaptation. In addition to the average size of the direct response increasing with the number of drivers, a higher proportion of regimes contained populations with a positive direct response to selection when more drivers were present (Fig. S2). This is unsurprising, because regimes with more drivers are more likely to contain a dominant driver, such that selection is strong enough to drive adaptation (7) (Fig. 2B-F, grey boxplots, Fig S3). Rather than constraining evolution, as predicted by the pleiotropy hypothesis, increasing the number of drivers in an environment leads to more adaptation due to stronger selection. Pleiotropic constraints may be present, but do not override the effects of stronger selection here.

A few dominant drivers affected the direct response to selection. These were reduced phosphate ($F_{1,329} = 26.197$, $P < 0.0001$), herbicide ($F_{1,334} = 7.862$, $P = 0.005$) and elevated CO_2 ($F_{1,346} = 7.83$, $P = 0.005$) (see Table S1 for regimes). The number of drivers in regimes explained less than one percent of the variation in the direct response to selection once the initial drop in growth rate and the identity of the drivers were taken into account (Table S5; $F_{1,329} = 0.89$, $P = 0.346$). The initial drop in growth rate and the identity of the selection regimes explained 27% of the variation in the direct response, but there was divergence among replicate populations within regimes, which explained most of the variation in the direct response of the evolved populations (40%).

Before evolution, the trait values for cell size and chlorophyll content showed a large plastic response to the multidriver environments, but the plastic response was fully or nearly reversed by the end of the evolution experiment. Because traits converged on similar values across regimes after ~450 generations, the majority of variation must be explained by variation between replicate populations. This variation, though statistically significant, is extremely low.

Following evolution, there was little variation in cell size when populations were grown in their own regimes (Fig. 3A triangles; $15.5 \mu\text{m} \pm 0.41 \mu\text{m}$; Mean \pm SD over all environments). The number of drivers in regimes explained less than two percent of the variation in cell size of the evolved populations, while variation among evolved populations within regimes (28%) and the identity of the environmental drivers (13%) explained most of the variation in cell size. Similarly, the positive relationship between cell size and population growth rates before evolution (Fig. S5A; $r^2 = 0.33$, $P < 0.0001$) broke down after evolution (Fig. S5B; $r^2 = 0.02$, $P = 0.006$). This suggests that the minimal variation in evolved cell size is neutral or near-neutral in terms of growth.

In this experiment, a proportion of the population often reversibly bleached (had no detectable chlorophyll autofluorescence using a flow cytometer) as a plastic response to regimes that reduced population growth rates. Before evolution, the proportion of chlorophyll positive cells (chlorophyll autofluorescence detectable using a flow cytometer) in populations decreased as the number of drivers in an environment increased (Fig. 3B circles; $F_{1,93} = 7.945$, $P = 0.0058$). In contrast, the number of drivers in an environment failed to explain variation in the proportion of chlorophyll positive cells after ~450 generations (Fig. 3B triangles; $F_{1,58} = 0.800$, $P = 0.375$). This is probably adaptive, as populations with higher proportions of chlorophyll positive cells had higher population growth rates (Fig. S6A; $r^2 = 0.47$, $P < 0.0001$; Fig. S6B; $r^2 = 0.24$, $P < 0.0001$), and photosynthesis is essential for rapid growth in media with no carbon additions (26). While reversible cell bleaching itself requires further study, the restoration of the capacity for photosynthesis is obviously adaptive here.

Prior to evolution, the chlorophyll concentration in cells depended on the number of drivers in the environment (Fig. 3C circles; $F_{1,93} = 24.676$, $P < 0.0001$). This trend is absent after evolution (Fig. 3C triangles; $F_{1,64} = 0.058$, $P = 0.811$), and chlorophyll autofluorescence per cell volume in evolved populations in their own regimes did not differ from that of evolved control populations in the control environment ($12.15 \pm 3.07 \text{ l}/\mu\text{m}^3$; mean \pm SD). This is consistent with populations having adapted to their environments, as they no longer show a standard sign of stress (27–29).

Discussion

To understand how the evolutionary response of primary producers depends on the identity and number of drivers in multidriver environments, we evolved microbial populations in ninety-six unique single and multidriver environments. Both the absolute growth rate of the evolved populations and the direct response to selection (the amount of evolution needed to reach that growth rate) were explained by the presence of a few dominant environmental drivers. Surprisingly, the multidriver context in which dominant drivers occurred had little effect on the growth rate of evolved populations on average. However, populations had a larger direct response to selection in environments that contained more drivers, indicating that primary producers evolve more in response to dominant drivers when they occur in multidriver environments than when they occur singly. This is largely because on average, selection is stronger in multidriver environments. Thus, populations evolving in multidriver environments adapt more, but arrive at the same final growth rates as populations evolving in single driver environments with the same dominant drivers.

We were initially surprised by these results. We had hypothesized that pleiotropic constraints would be more important in populations evolving in environments with more drivers because more traits would be under selection (30). Instead, the response to selection increases with the number of environmental drivers. Above a threshold number of drivers (seven, in this experiment), rapid adaptation was not possible, and populations went extinct. These results suggest that the number of traits under selection does not scale with the number of environmental drivers. This makes sense given that evolutionary responses are driven by a few dominant drivers (CO_2 , low phosphate, temperature, and herbicide), so that the traits under selection may be more or less constant over the regimes containing a given dominant driver. As the number of drivers increases, dominant drivers are more likely to be present. The identity of dominant drivers in each regime partially explains the small differences in the response to selection in multidriver environments. The overriding effect of the dominant drivers is consistent with the acclimation response

to these regimes (7), scenario-based experiments (8), and many physiological responses of microalgae to pairs of drivers (31–35). Our data are consistent with either small effects of driver interactions relative to the effects of dominant drivers, or of (nearly) zero-sum interactions among drivers.

Our findings highlight the importance of accurately gauging the intensity of selection for understanding the evolutionary potential of primary producers. Predicting the intensity of selection that a population is likely to experience requires knowing first, which drivers are present locally, and second, the organismal responses to the dominant drivers. Since populations experience their local environment rather than a global average, this requires using regional rather than global patterns of multidriver change (5). The regimes in this experiment did not reflect realistic environments, nor were they intended to – the experiment was carried out using a laboratory model system and 96 different environments, with the goal of disentangling the roles of the number and identity of environmental drivers on evolution over hundreds of generations. In addition, functional groups (e.g. calcifiers, silicifiers, nitrogen fixers) of primary producers respond differently to dominant drivers (4, 36, 37). Our results also suggest that it could also be useful, when considering how primary producers may evolve under different climate change clusters (5), to group drivers based on their effects on growth (positive, negative, neutral) for different taxa when assessing whether or not we expect climate change clusters to drive evolution.

Our approach complements scenario-based studies. For example, Boyd et al (5) modeled regional changes to multidriver regimes, and used measured shifts in phytoplankton physiology to make qualitative predictions about shifts in biogeography. They detail the responses of coccolithophores (calcifiers) and diatoms (silicifiers) in two high latitude ocean provinces to shifts in temperature, CO_2 , photosynthetically active radiation (PAR), iron, silicate, nitrate and phosphate, as well as interactions between driver pairs. Based on plastic responses, they suggest that elevated temperature is likely to cause a poleward shift in coccolithophores, and high PAR, low phosphate, and low silicate are likely to favor coccolithophores over diatoms. A decrease in calcification is predicted for coccolithophores and a decrease in silicification for diatoms. Our study suggests that in addition, selection to increase both calcification and silicification could drive evolution in multidriver environments. We also expect that evolution reverse some of the trait change predicted based on the Boyd et al (5). Adaptation could also result in (positive and negative) changes in growth rates eventually attenuating, based on data from single-driver evolution experiments (1, 2). This illustrates how we can use an understanding of how natural selection acts to refine predictions of trait change in multidriver environments.

Despite some populations having a large direct response to selection, evolution reversed plastic changes in several traits. This suggests that as populations adapt to multidriver environments, the function of evolving groups may change less than expected based on plastic responses to multidriver environments (plastic responses are circles in Fig. 3). Population growth rates, however, are not restored to control values in all regimes. Since the populations in this experiment are propagated by batch culture and were not allowed to reach carrying capacity, overall selection was for increased in cell division rates (20). The lower growth rates in some regimes may be the result of physiological constraints, since resources can limit growth rates even after adaptation. For example, growth rates are lower in low phosphate regimes than in phosphate replete ones regardless of driver number (Fig. S4). Despite this, the proportion of cells showing signs of stress (chlorophyll negative and/or small cells) in evolved populations in the low phosphate and low-nutrient environments returned to control values (Fig. 3). In contrast, populations evolved in

the control environment produce small cells and high proportions of chlorophyll negative cells when grown in low nutrient environments. Both the proportion of chlorophyll positive cells and cell size are correlated with population growth rates, but this correlation is much weaker in evolved populations, largely because those traits have converged. This indicates that cells adapted to tolerate the low nutrient multidriver environments, even if they lack the resources to increase growth rates. Similarly, in environments with seven drivers, populations have low growth rates, but normal chlorophyll content and cell sizes. This is in line with other studies that demonstrated that in poor-quality environments populations invest in maintaining cell condition (38, 39). In *C. reinhardtii*, cell size is related to population growth through its effect on cell division, as a critical size must be reached before cells divide (40–42). This trend of phenotypic reversion during adaptive evolution has also been seen in high CO₂ environments (43), indicating that some phenotypic reversion may be a common outcome of evolution (44).

We did not examine the molecular basis of trait reversion here, but offer two non-exclusive explanations. First, our results are consistent with compensatory mutations affecting trait evolution in multidriver environments. Following a large drop in population fitness, the first beneficial mutation fixed often has a large effect (45), and can change several traits simultaneously. This may be followed by compensatory mutations that increase fitness by reversing the effect of the initial mutation on traits where change was not adaptive (46–48). Second, heritable epigenetic mutations or transgenerational plasticity can contribute to early adaptation (7), but eventually be replaced by genetic mutations (49–52). Understanding how trait reversion is linked to adaptation presents an opportunity to improve our predictions of functional trait values in primary producers in aquatic environments.

Laboratory evolution experiments use simplified environments and populations to gain insights into the fundamental action of natural selection. Applying the results of this experiment to natural phytoplankton population requires taking into account how population size and diversity (among other factors, such as recombination rates) affect adaptation (53). Previous work has shown that higher standing genetic variation can allow adapting populations to evolve faster (54–57). Similarly, recombination and plasticity (1, 58) can both speed up adaptation (59). The power of simplified laboratory experiments lies in providing insight into how natural selection works; applying these insights requires accounting for other processes that can modify evolution, and for the specific biology of wild populations.

Conclusions

We show that populations adapt more in response to dominant drivers when those drivers occur in a multidriver context, until environments deteriorate enough to cause extinctions. Alongside this, adaptation can result in the reversion of the initial changes to trait values in multidriver environments. While we expect that the result of evolution being driven primarily by a few dominant drivers in multidriver environments to be general, the identity of dominant drivers will be organism and context dependent, such that a variety of approaches (large factorial experiments like this one, scenario-based models and experiments, taxa-specific physiological and evolution studies) are needed to understand how primary producers will respond to multiple environmental drivers. In addition, the evolutionary potential of populations will depend on demography, existing genetic variation and the rate at which new variation can be generated by recombination, migration and the availability of spatial and temporal refugia. Our results emphasize the importance of gauging the intensity of selection on populations under global change (60) by linking complex environmental change to organismal fitness. This informs our understanding of the extent to which primary pro-

ducers evolve in multidriver environments. A second challenge is understanding the evolutionary reversion of plastic responses in functional traits, as this will determine the function of primary producers in under complex environmental change.

Methods and Materials

Selection experiment

All populations were founded from one colony originating from a single cell of *C. reinhardtii* (CC-2931, mt-; Chlamydomonas Resource Centre, University of Minnesota), grown in sterile Sueoka's high salt medium with Tris HCl (HSMT; (61)) (Table S2, Table S3). The ancestral population was split into 576 populations (Figure 1, Table S1). A single founder colony ensures that population evolution uses *de novo* variation. Environmental changes occurred in one step at the beginning of the experiment (control level in brackets): increased CO₂ to 2000ppm (420ppm), temperature to 26°C (25°C), decreased pH to 6.5 (7.2 pH), light levels to 18 μmol m⁻² s⁻¹ (32 μmol m⁻² s⁻¹), reduced phosphate to 1.69 mM (13.56 mM), nutrient depletion to 25% (100% nutrients Table S3), 0.5 μM of atrazine (no herbicide). Regimes with UV were dosed with 8.1 KJ.m⁻² UVB twice a week (Table S4). Populations evolved in environments with at least one driver are multidriver-evolved populations. Populations evolved in the control environment are control populations. All populations were propagated by batch culture. See SI Appendix for details.

Assays of population growth rates

An acclimation period of one transfer cycle was used for all assays (see SI).

The average rate of cell division per day was calculated over a single batch culture-length time using equation 1(7). See SI Appendix for details.

$$\text{Rate of Division } (day^{-1}) = \frac{\left(\log_2 \left(\frac{N_t}{N_0} \right) \right)}{(t_f - t_0)} \quad (1)$$

N_t is the cell density (cells/ ml) at time t (hours) and N_0 is the cell density at time t_0 .

The direct response to selection was measured by comparing the growth of a multidriver-evolved population and a control population in the same multidriver regime (Fig. 1). The direct response to selection was calculated using equation 2, and scaled to the number of divisions (d^{-1}) of the control population in the relevant regime. See SI Appendix for details.

$$s = \frac{(E - C)}{C} \quad (2)$$

E is the number of divisions (d^{-1}) of multidriver-evolved populations in their regimes, and C is the number of divisions (d^{-1}) of evolved control populations in the same regimes.

Flow cytometric analysis of physiological parameters

An acclimation period of one transfer cycle was used for all assays, as above. A FACS CANTO was used to determine red autofluorescence (chlorophyll a and b), event number (cell density) and forward scatter (cell size) (1, 62). See SI Appendix.

Statistical analysis

The effects of i) driver identity and ii) the plastic response on the response to selection were analysed with a mixed model in R (63), using the packages lme4 and lmerTest. The plastic response and the presence/absence of drivers are fixed factors. The effects of i) the number of drivers and the ii) identity of the drivers on evolved growth rate were also analysed using a mixed model. The number of drivers (0-7) and driver identity (e.g. CO₂) are fixed factors. Regime identity (Table S1), batch number and replicate populations within regime are random factors (SI Appendix for details).

The contribution of fixed factors was estimated using equation 4 (as described in (7)),

$$\text{Percentage of Fixed Effect Variance} = \left(\frac{\sigma_F^2 \times (b^2 - se^2)}{\sigma_x^2} \right) \times 100 \quad (4)$$

where σ_F^2 is the variance of the fixed effect, b is the slope of the fixed effect, se is the standard error of the fixed effect, and σ_x^2 is the variance of the response variable.

Supporting data and R code will be archived in Dryad (include doi here).

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